Although eimeriid coccidia are generally site specific for the intestinal tract, some species that infect mammals and birds are also known to be capable of developing in organs other than the intestine. *Eimeria stiedai* normally occurs in the liver of the rabbit and *E. truncata* in the kidneys of geese and ducks (Overstreet, 1981). The coccidians, *E. gruis* and *E. reichenowi*, are common parasites of whooping cranes and sandhill cranes, which are associated with granulomatous necrotizing hepatitis, pneumonia, and splenitis (Carpenter et al., 1980; Novilla et al., 1981). One of these species is likely the cause of visceral coccidiosis in this case, but fecal examination was not performed, so the organism could not be further identified.

To our knowledge, this is the first report of *Cryptosporidium* sp. infection in cranes. Cryptosporidiosis is often associated with an immunosuppressed state (Tzipori, 1988), so immunosuppression may have contributed to the systemic spread of the eimerian parasite in this case. However, there was no conclusive evidence of immunosuppression in this bird. Alternatively, the eimerian infection may have caused immunosuppression allowing *Cryptosporidium* sp. infection in this crane.

Two other cranes that had been kept in the same exhibits died about 1 mo before submission of this case. According to the attending veterinarian, although coccidia were identified as *Eimeria* spp. on fecal floatation, the species could not be determined. The remaining live cranes in the exhibit were treated with a coccidiostatic drug, and no clinical coccidiosis has been noted since.

Disseminated visceral coccidiosis is an occasional cause of mortality in wild and captive cranes. Management of disseminated visceral coccidiosis in cranes is crucial to prevent fatality. This study indicates that anticoccidian programs directed against visceral coccidiosis should be undertaken in zoos.

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Significant Morphological but Little Molecular Differences Between *Trypanosoma* of Rodents From Alaska

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ABSTRACT: We examined blood smears of 173 rodents and 33 shrews captured at 4 sites in the Gates of the Arctic National Park, northern Alaska, in summer 2002. *Trypanosoma* spp. were detected in the plasma of 5 *Microtus oeconomus*, 4 *Microtus miurus*, and 1 *Lemmus trimucronatus*. The trypomastigote morphology from different individuals of *M. oeconomus* caught at the same site and of *M. miurus* from different sites varied significantly. The 4 DNA sequences obtained from the blood smear positive samples contained 2 different haplotypes very similar to each other and to that of *Trypanosoma microti*. Of possible vectors of blood parasites, the flea *Amalaraeus dissimilis* was collected from *M. miurus*.

Despite the increasing interest for biodiversity assessments, baseline information on the parasite diversity in the Arctic and subarctic regions is minimal or absent for many mammal species. The Beringian Coevolution Project (BCP) was designed as a comprehensive inventory of mammals and parasites across Beringea to document the effects of historical and contemporary global changes on faunal assemblages in Arctic Region (Hoberg et al., 2003). As a part of this project, a wide array of parasite samples were collected from small mammals in the Gates of the Artic National Park during summer of 2002. In this article, we describe the *Trypanosoma* spp. infections found in rodents during that survey and compare the trypomastigote morphology with those recorded from arctic rodents in previous studies. We also provide molecular data from 3 *Trypanosoma*-infected rodent species.

The material consisted of blood smears of 173 rodents and 33 shrews captured at 4 sites in the Gates of the Arctic National Park, Alaska, U.S.A, in July and August of 2002. The number and species caught are shown in Table I. Three of the sites (Agiak Lake, 68°04′48.4″N, 152°56′44.1″W, altitude 960 m above sea level; Fortress Mountain, 68°34′48.4″N, 152°57′35.6″W, altitude 750 m; and Nanushuk River, 68°16′25.4″N, 150°39′17.6″W, altitude 990 m), are arctic tundra habitat. The unnamed lake site (67°27′17″N, 150°51′49.5″W, altitude 320 m) North of Koyukuk River is taiga forest habitat close to the tree line. Animals were caught with snap-traps (Museum Special and the Finnish Type) and plastic pitfalls. The locations of all trapping lines were recorded by global positioning system. Each animal was assigned a permanent archival record number (AF in appendices), and voucher specimens (skeletons, skulls, skins, and frozen tissues) were deposited in the University of Alaska Museum at Fairbanks. Animals were dissected immediately after capture in the field. On necropsy, animals were combed for ectoparasites, which were stored in 70% ethanol for later identification. A drop of blood was obtained from the heart for preparation of thin blood smears, which were air-dried, fixed in methanol, and stained later in the laboratory with Giemsa's stain. Each smear was examined with a microscope for 5 min at ×400 and for 10 min at ×1,000.

DNA was extracted from 10 blood smear positive samples collected on Isocode® STIX (Schleicher and Schuell, Keene, New Hampshire) using an alkaline digest method (Bown et al., 2003). In brief, the blood-stained Isocode® blotting papers were boiled at 100 C in a 500 μl solution of 1.25% NH_3 on a dry hot plate in 1.5-ml Sure-Lock microcentrifuge tubes (Fisher Scientific, Loughborough, U.K.). After 20 min, tubes were removed, centrifuged briefly, and replaced with the lid open for a further 25 min, or until half of the original volume remained. A nested polymerase chain reaction (PCR) was used which specifically targets a variable region of the *Trypanosoma* 18S ribosomal RNA (rRNA) gene (Noyes et al., 1999, 2000). Each 50-μl reaction contained 5 μl of a 1:10 dilution of the DNA solution, 25 μl 2× PCR Master Mix (Abgene, Surrey, U.K.), 18 μl ddH_20, and 1 μl each of the following external primers: TRY927F 5′ GAAACAAGAAACACGGGAG and TRY927R 5′ CTACTGGGCAGCTTGGA, for 30 cycles of 94 C 30 sec; 55 C 60 sec; 72 C 90 sec. The products from the first-stage reaction were diluted 1:10 and 2 μl used as a template for the second

TABLE I. Hemoparasites of small mammals caught at 4 sites at the Gates of the Arctic National Park, Alaska, in 2002. N = number of blood smears examined.

	N	*Trypanosoma* spp.	*Bartonella* spp.
Agiak Lake 68°04′50.9″N, 152°56′31.4″W			
Microtus oeconomus Pallas	18	4	3
Microtus miurus Osgood	18	0	0
Lemmus trimucronatus Davis	6	1	3
Clethrionomys rutilus Pallas	16	0	3
Sorex monticolus Merriam	22	0	1
Unnamed lake, Koyukuk 67°27′17″N, 150°51′49.5″W			
Microtus oeconomus	2	0	0
Microtus miurus	1	1	1
Lemmus trimucronatus	12	0	6
Clethrionomys rutilus	16	0	2
Synaptomys borealis Richardson	31	0	3
Sorex cinereus Kerr	1	0	0
Sorex hoyi Baird	5	0	0
Fortress Mountain 68°34′48.4″N, 152°57′35.6″W			
Microtus oeconomus	3	1	2
Microtus miurus	28	2	10
Dicrostonyx groenlandicus Traill	3	0	0
Sorex monticolus	1	0	0
Sorex cinereus	3	0	0
Sorex yukonicus Dokuchaev	1	0	0
Nanushuk River 68°16′38.5″N, 150°39′19.6″W			
Microtus oeconomus	4	0	0
Microtus miurus	15	1	4

round using 1 μl of the following internal primers: SSU561F 5′ TGGGATAACAAAGGAGCA and SSU561R 5′ CTGAGACTGTAACCTCAAAGC, 1 μl dd$H_2$0, 45 μl ReddyMix® PCR Master Mix (Abgene) and the same cycling conditions as outlined above. DNA extraction, primer preparation, and the first- and second-stage PCR preparation were all carried out in separate dedicated rooms to reduce the risk of contamination.

Twelve microlitres of the second-round PCR product were loaded onto a 1.5% agarose gel stained with ethidium bromide and observed under UV light. Samples producing a band of ~540 kb were considered positive (AF 59919; AF 61877; AF 61380; AF 59915). Amplified DNA was purified with a QIAquick PCR Purification Kit (QIAGEN, Sussex, U.K.) according to manufacturer's instructions and sequenced commercially (ABC, Imperial College, London, U.K.) using an ABI 377 automated sequencer.

Trypomastigotes were detected in the blood smears of 5 *Microtus oeconomus*, 4 *Microtus miurus*, and 1 *Lemmus trimucronatus* (Table I). All infected *M. oeconomus* from Agiak Lake were mature females (2 were pregnant). The 2 infected *M. miurus* from Fortress Mountain were immature males. The rest were all mature males. The number of hosts was too low for statistical comparisons between sex and age groups. The number of trypomastigotes found in the plasma of the infected rodents ranged from 3 to 22 per 1,000 erythrocytes. In the infected *L. trimucronatus*, only 5 trypomastigotes were found, even after examination of the entire smear. In a few samples, the morphology of the trypomastigotes was poorly preserved most likely because the blood smears had not dried properly in the wet field conditions. Trypomastigotes from 7 infected animals were measured (as in Laakkonen et al., 2002) for morphological comparison (Voucher specimens in U.S. National Parasite Collection, USNPC nos. 94139, 94140, 94141, 94142, 94143, 94144, 94145).

The mean total length of trypomastigotes from *M. oeconomus* ranged from 21.85 to 26.5 μm and that of the flagellum from 4.35 to 6.50 μm. In *M. miurus* the corresponding values varied from 22.8 to 29.8 μm and from 3.45 to 6.80 μm. The detailed measurement data of the 9 trypomastigote parameters measured are available from the corresponding author on request.

The trypomastigote morphology from different individuals of *M. oeconomus* caught at the same site (Agiak Lake) varied significantly ($P < 0.001$, not shown). In *M. miurus*, similar, significant variation in the trypomastigote morphology was detected between vole individuals from different sites ($P < 0.001$, not shown). The trypomastigotes of *L. trimucronatus* were large (maximum total length 36 μm), and had a relatively long flagellum (mean 11.35) compared with that of trypomastigotes from other host species.

The trypomastigotes found in *M. oeconomus* were larger than those seen in this host species from Toolik Lake, Alaska (Laakkonen et al., 2002), but similar in size and other morphological characteristics to trypomastigotes of *M. oeconomus* from Lower Ugashik Lake, Alaska Peninsula (Fay and Rausch, 1969). The trypomastigotes of lemmings appear to be slightly larger, and to have longer flagella than those of *Microtus* spp. voles (Quay, 1955; this study). Our *Trypanosoma* sp. findings in *M. miurus* and *L. trimucronatus* appear to be new host records (Podlipaev, 1990).

The considerable differences in the morphology of trypomastigotes from the same host species within, and between, study sites indicates that morphological comparison alone is not a reliable method for the identification of *Trypanosoma* species of arctic rodents. This result supports the conclusions of the only previous comprehensive biometrical comparison of *T. lewisi*–like trypomastigotes, revealing significant variations in size between parasite populations from the same host and no significant differences between parasite populations from different host species (Davis, 1952). Other blood parasites (*Babesia* spp.) are known to be morphologically pleomorphic depending on the host species or stage of infection (Kreier and Baker, 1987).

The 18S rRNA haplotypes from the *M. miurus* (AF 61877 and AF 61380) differed from that of *Trypanosoma microti* at 2/15 polymorphic positions (not shown). The haplotypes from the *L. trimucronatus* (AF 59915) and *M. oeconomus* (AF 59919) differed from that of *T. microti* at 1/15 polymorphic positions and from that of the *M. miurus* at 3/15 positions (GenBank AY 586621, AY 586622, AY 586623, and AY 586624).

The sequence data obtained from the 3 host species caught at 3 different locations showed that the *Trypanosoma* spp. found from different rodent species were very similar to each other and to that of *T. microti*. However, the European *Trypanosoma* species *T. microti* and *T evotomys* differ from each other at only 2/15 polymorphic positions (Noyes et al., 2002), consequently the small differences between haplotypes may be markers for significant biological differences in these parasites. Nevertheless, the data indicated that the haplotypes did not correlate with morphological types. Additional molecular analyses are needed to determine whether the identical haplotypes found in *L. trimucronatus* and *M. oeconomus* (caught at the same site) can be taken as evidence for possible geographic populations.

The record of *Trypanosoma* sp. in Alaskan rodents consists of only a few reports on wild rodents (Quay, 1955; Fay and Rausch, 1969; Laakkonen et al., 2002) and of 1 on the introduced *Rattus norvegicus* (Schiller, 1956). Of wild rodents, trypanosomes have been detected in a few *Dicrostonyx torquatus* (Quay, 1955) but not in any of the few *Microtus pennsylvanicus* and *Synaptomys borealis* examined for blood parasites (Quay 1955; Laakkonen et al., 2002; Table I). Trypanosomes have not been reported from arctic shrews, but sample sizes in this and in previous, similar studies have been low (Laakkonen, 2000; Laakkonen et al., 2002).

Rod-shaped organisms resembling *Bartonella* sp. bacteria were detected in all examined rodent species, except *Dicrostonyx groenlandicus* (Table I), which had a low sample size (N = 3). Because several morphologically similar microorganisms are found inside, or on the surface of red blood cells of wild rodents, definite identification was not attempted based on morphology.

Of the *Trypanosoma* sp.–infected rodents, fleas were found only in 1 *M. miurus* from the unnamed lake site at Koyukuk. It had 2 females

of *Amalaraeus dissimilis* in it. This old male vole was also infected with the *Bartonella* sp.–like rods in erythrocytes.

The number of ectoparasites found in the infected hosts was low at least partly because the collections were conducted on dead hosts. Ectoparasites tend to leave the host shortly postmortem, once the core temperature drops to ambient levels. The present information on ectoparasite fauna of arctic rodents (Holland, 1985; Murrell et al., 2003) indicates that although some ectoparasite species may be primarily associated with certain host species, many arctic rodent species share several ectoparasite species. This provides interesting avenues for the transmission of *Trypanosoma* spp. and other blood parasites of these, and other hosts (Noyes et al., 2002). Further molecular analyses of *Trypanosoma* spp. from both fleas, and their host species are needed to gain insight into the host–parasite coevolution of hemoparasites of (arctic) small mammals.

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Genotyping of *Giardia duodenalis* From Humans and Dogs From Mexico Using a β-Giardin Nested Polymerase Chain Reaction Assay

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ABSTRACT: Cysts of *Giardia duodenalis* were collected in Mexico from symptomatic children (n = 9) and from pet dogs (n = 5), and they were directly characterized by nested polymerase chain reaction (PCR) amplification of the β-giardin gene. Eight isolates of human origin established as in vitro cultures and 2 reference strains, representing assemblages A and B of *G. duodenalis*, were also analyzed. PCR–restriction fragment length polymorphism showed that all isolates belonged to assemblage A. Sequence analyses indicated that the large majority of isolates were of the A1 genotype; interestingly, 2 human isolates displayed the A3 genotype, which has been previously identified in human isolates from Italy. The presence of cysts of the A1 and A3 genotypes in isolates from pet dogs is consistent with their role as reservoirs for human infection, although further studies are needed to confirm the occurrence of zoonotic transmission. Remarkably, cysts of assemblage B have not been found in any of the Mexican isolates studied to date.

Giardia duodenalis (syn. *Giardia intestinalis*, *Giardia lamblia*) is an enteric protozoan parasite, which infects a wide variety of mammals, including humans. Individuals with acute infection generally present with diarrhea, abdominal pain, nausea, and weight loss, although the infection is asymptomatic in a significant proportion of cases (Faubert, 2000).

Giardiasis is 1 of the leading causes of epidemic gastroenteritis worldwide. In Africa, Asia, and Latin America, approximately 200 million people have symptomatic giardiasis, with some 500,000 new cases per year (WHO, 1996). With specific regard to Mexico, epidemiological data suggest that giardiasis is the most common enteric parasitic infection, with the highest prevalence observed among children (Sotelo-Cruz, 1998), a finding related to the immature immunological status of children and poor hygiene practices (Thompson, 1994).

Despite their morphological homogeneity, *G. duodenalis* isolates from different mammalian species display considerable genetic variability, and at least 7 groups, or assemblages (designated as "assemblages A–G"), have been described (Monis et al., 2003). Assemblages